PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

FILOGEOGRAFIA DO LEÃO-MARINHO-DO-SUL, *OTARIA FLAVESCENS* SHAW 1800

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Marcelo Coelho Miguel Gehara
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Porto Alegre, fevereiro de 2009

"Sei que a arte é irmã da ciência
Ambas filhas de um deus fugaz
Que faz num momento e no mesmo momento desfaz..."
Gilberto Gil

ÍNDICE

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Quero agradecer a oportunidade antes de tudo. Foi muito prazeroso realizar este trabalho, aprender com as dificuldades e os desafios que apareceram no caminho. Antes e durante o trabalho várias pessoas foram importantes, cada uma responsável por uma parte. Quando as várias partes se juntam, mesmo sem saber que estão se juntando o todo se torna possível.

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Se sente seguro aquele que sabe que tem o amor incondicional de alguém. Se sente forte aquele que tem por alguém amor incondicional. Eu me sinto seguro e forte.

Andréia

A vida é muito mais legal do que eu pensava. Muito mais bonita, motivante, inspirativa e prazerosa.

Sinto que aprendi muito, mesmo sabendo muito pouco, sinto-me feliz e mais esperto, sinto-me preparado para o próximo.
Dedico este trabalho ao próximo!

RESUMO

Neste estudo investigamos a estrutura populacional do leão-marinho-do-sul (Otaria flavescens), um otarideo amplamente distribuído ao longo das costas Atlântica e Pacífica da América do Sul, e que foi extremamente caçado durante os dois últimos séculos. Apesar de sua ampla distribuição e interações com atividades de pesca, até o momento poucos trabalhos avaliaram as diferenças genéticas e estruturação ao longo da distribuição da espécie. No presente trabalho, utilizamos marcadores de microssatélites (10 loci) e DNA mitocondrial para avaliar a estrutura populacional e história evolutiva da espécie. Encontramos estruturação significativa entre as populações dos oceanos Pacífico e Atlântico, correspondendo a duas linhagens mitocondriais reciprocamente monofiléticas, separadas desde o início do Pleistoceno, indicando forte filopatria das fêmeas. Também encontramos estruturação genética significativa intra-oceânica entre diferentes sítios de reprodução. A análise dos microssatélites também demonstrou que as populações dos dois oceanos são significativamente diferentes, possuindo diversos alelos exclusivos, apesar de que um pequeno fluxo gênico inter-oceânico através dos machos não pode ser descartado. Nossos dados mostram que a espécie não sofreu recentemente nenhuma redução significativa na sua diversidade genética. Estes resultados indicam fortemente que as populações de *O. flavescens* do Pacífico e do Atlântico são duas unidades evolutivas significativas (ESUs) e que as colônias de reprodução em cada oceano devem ser manejadas separadamente.

ABSTRACT

We investigated the population structure of the Southern sea lion (*Otaria flavescens*), an otariid widely distributed along the Pacific and Atlantic coast of South America, which was heavily hunted during the two last centuries. Despite its wide distribution and interactions with fishing activities, few works evaluated the genetic differences and structuring along the species distribution. Here we used both microsatellite (10 loci) and mtDNA markers to evaluate the population structure and evolutionary history of the species. We found significant structuring between Pacific and Atlantic populations that corresponds to two reciprocally monophyletic mitochondrial lineages separated since early Pleistocene, indicating extreme female phylopatry. We also found significant genetic structure between intra-oceanic breeding sites. Microsatellites analyses also found the populations from the two oceans as significantly different with several private alleles, although very small inter-oceanic gene flow mediate by males could not be discarded. Our results show that the species did not suffer recently any significant reduction of its genetic diversity. Our findings strongly support that *O. flavescens* Atlantic and Pacific populations are two evolutionary significant units (ESUs) and that intra-oceanic breeding colonies should also be managed separately.

APRESENTAÇÃO

O leão-marinho-do-sul, *Otaria flavescens* Shaw 1800, é um dos otariídeos mais amplamente distribuídos ao longo da América do Sul, possuindo colônias reprodutivas tanto na costa Pacífica, desde o sul do Peru até o extremo-sul do Chile, como na costa Atlântica a partir do sul da Argentina até o sul do Brasil (Vaz-Ferreira, 1982), (**Figura 1**). Contudo, registros de indivíduos fora da sua área normal de distribuição são relativamente comuns. Para a costa brasileira há ocorrência em Santa Catarina (Simões-Lopes *et al.* 1995), Paraná, São Paulo e Rio de Janeiro (Pinedo, 1990). Já na costa do oceano Pacífico existem registros para a Colômbia (Mora-Pinto & Muñoz-Hincapie, 1995; Capella *et al*., 2002) e Ilhas Galápagos (Wellington & de Vries, 1976), alcançando até mesmo a costa do Panamá na América Central (Mendez & Rodriguez, 1984).

Apesar do Brasil não possuir nenhuma colônia reprodutiva de lobos ou leões-marinhos, muitos espécimes podem ser observados anualmente nas costas sul e sudeste, entre os meses de outono e primavera quando realizam seus deslocamentos pós-reprodutivos auxiliados pela corrente das Malvinas (Repenning *et al.,* 1971; Carvalho, 1975; Oliveira 1999).

Não existem estimativas populacionais atualizadas precisas da espécie ao longo da América do Sul devido à ausência de estudos recentes. Contudo, estima-se que existam aproximadamente 265.000 espécimes, dos quais 90.000 estariam na Argentina, 3.000 nas Ilhas Falkland, 12 a 15.000 no Uruguai, 100 no Brasil, 5.000 no Peru e 128.000 no Chile (Seal Conservation Society, www.pinnipeds.org).

Esta espécie foi intensamente caçada até o início da década 90, principalmente nas áreas reprodutivas da costa Atlântica, como o Uruguai (Vaz-Ferreira & Bianco, 1998), onde sua exploração

comercial era tida como uma das bases da economia nacional. Centenas de milhares de espécimes do leão-marinho foram mortos entre os séculos XIX e XX, e como resultado várias populações foram quase levadas à extinção. Apesar da proibição da caça em todos os países de sua ocorrência, existem relatos de caça ilegal recente na costa do Peru e de mortalidade da espécie em decorrência da forte interação com atividade de pesca, sendo este atualmente o maior problema de conservação enfrentado pela espécie na América do Sul (Ott *et al*., 1996; Crespo & Dans, 2005).

Uma das maiores polêmicas com relação ao leão-marinho-do-sul é sobre o seu nome específico, ora denominado *Otaria byronia* (de Blainville, 1820), ora *Otaria flavescens* (Shaw, 1800). Os autores têm divergido fortemente, principalmente devido às dificuldades inerentes à busca da identidade correta da espécie devido à inexistência dos holótipos, os quais foram destruídos durante a segunda guerra mundial (King, 1954). Cabrera (1940) argumentou que a descrição original de *O. flavescens* feita por Shaw (tendo como nome original *Phoca flavescens*) com base em material coletado por Pennant no ano de 1793 corresponde a uma pele preparada de um indivíduo juvenil e de coloração amarelada. Apesar da localidade tipo do espécime descrito por Shaw ter sido o Estreito de Magalhães, local onde regularmente existem populações da espécie, não existe até hoje uma identificação positiva e inequívoca para esta pele, uma vez que outros otariídeos também ocorrem regularmente naquela região como o lobo-marinho sul-americano, *Arctocephalus australis*. Além disso, eventualmente pode haver ocorrência de outros lobos-marinhos como *A. gazella* e *A. tropicalis*, este último sabidamente de coloração ventral amarelada, muito semelhante à descrita por Shaw (1800).

Oliva (1988) foi categórica ao afirmar que o epíteto "*flavescens*" está baseado em um "arctocefalíneo não-identificável", e que o holótipo descrito por Shaw não corresponde a um filhote de *Otaria* no comprimento total, no tamanho da orelha e na já referida coloração do pêlo.

Rodriguez & Bastida (1993) apresentaram um conjunto de idéias relacionadas ao comprimento do corpo, tamanho da orelha, coloração e comprimento do pelo que confirmam que o holótipo de Shaw 1800 pode ser um filhote de Leão-Marinho-do-Sul, portanto o epíteto "*flavescens*" seria válido. Os autores argumentam que os filhotes de *Otaria* podem apresentar após a primeira muda uma fase de pelagem mais "clara" que em alguns indivíduos pode ser considerada "amarelada", o que jamais acontece com juvenis de *A. australis*. Vaz-Ferreira (1984) compartilhou dessa opinião apresentando outros casos de pêlos de filhotes de coloração clara e até mesmo a possível ocorrência de albinismo, embora muito rara. Rodriguez & Bastida (1993) consideram "muito improvável" a possibilidade de que se tratasse de um filhote de outro arctocefalíneo como *A. gazella*.

Já o holótipo de *Otaria byronia,* apesar de ter sido destruído, se tratava de um crânio sem mandíbula da espécie coletado pela expedição do comodoro John Byron que navegou pelo mundo no final do século XVIII. Este crânio foi depositado no Museu do *Royal College of Surgeons*, em Londres, sob o número 974 (Hamilton, 1934). Blainville foi quem o descreveu originalmente denominando-o de *Phoca byronia*, tendo incluído junto com a descrição um desenho mostrando claramente a extensão do palato secundário, característica diagnóstica dessa espécie. Além do palato expandido a ilustração mostra claramente as cristas nucais e sagital bem desenvolvidas (King, 1954), características essas que, aliadas ao tamanho (aproximadamente 33 cm), remetem a um indivíduo macho adulto de *Otaria*. Hamilton (1934) referiu-se ao espécime como "pertencente a um macho adulto" e, mesmo tendo sido sucinto na sua referência ao espécime sua identificação é confiável, pois ele viu pessoalmente o crânio antes que esse se perdesse durante a Segunda Guerra Mundial. No entanto, a localidade tipo para *Phoca byronia* foi referida como de Ilha Carolina, no Oceano Pacífico, local onde sabidamente não ocorre nem essa, nem nenhuma outra espécie de otariídeo. É sabido,

entretanto, que a mesma expedição do comodoro John Byron explorou o Estreito de Magalhães, e posteriormente, percorreu a referida região do oceano Pacífico. Tal fato da margem um possível erro de catalogação do espécime (Rice, 1998).

Recentemente a Comissão Internacional de Nomenclatura Zoológica se pronunciou a respeito, publicando em seu boletim (ICNZ, 2000) um parecer (Opinião 1962) onde foi regulamentado como válido o epíteto específico "*byronia*" para a espécie *Otaria byronia*. Entende-se por regulamentação a inclusão do nome na Lista oficial de nomes específicos em Zoologia (Opinion, 1962). Essa decisão foi baseada no caso 3058, também publicado no Boletim de Nomenclatura Zoológica e de autoria de Gardner & Robbins (1999).

Os pesquisadores argentinos, Diego Rodriguez & Ricardo Bastida (2008), apresentaram recentemente um pôster na XIII Reunión de Trabajo de Especialistas em Mamiferos Acuáticos de América Del Sur, 7º Congreso SOLAMAC, argumentando a favor do epíteto *flavecens.* No referido trabalho os autores invocam o principio da prioridade (o nome *flavescens* é de 1800, enquanto que o nome *byronia* é de 1820). Eles argumentam que para aceitar o segundo é preciso rechaçar completamente o primeiro, o que de fato ainda não aconteceu, uma vez que nenhum elemento novo (fora os tradicionais argumentos sobre coloração de pêlo, localidade-tipo e todos os outros já apresentados anteriormente) acerca da invalidade destes nomes foi acrescentado. No presente estudo adotamos *Otaria flavescens* como o nome válido para o Leão-marinho-do-sul em acordo com o principio da prioridade e com os argumentos de Rodriguez & Bastida (2008).

Apesar da ampla distribuição geográfica, da caça indiscriminada da espécie, dos problemas de interação com a pesca, raros foram os estudos que buscaram avaliar as possíveis diferenças

populacionais e suas conseqüências para o manejo e conservação da espécie ao longo da América do Sul.

Desta forma, este trabalho teve a intenção de avaliar possíveis diferenças genéticas ao longo da distribuição do Leão-Marinho-do-Sul, buscando entender sua história demográfica e os possíveis eventos e fatores que influenciaram no padrão filogeografico da espécie.

Phylogeography of the Southern sea lion, *Otaria flavescens* (Otaridae)*:* early Pleistocene divergence between Atlantic and Pacific populations

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Running title: Phylogeography of the Southern sea lion

ABSTRACT

We investigated the population structure of the Southern sea lion (*Otaria flavescens*), an otariid widely distributed along the Pacific and Atlantic coast of South America, which was heavily hunted during the two last centuries. Despite its wide distribution and interactions with fishing activities, few works evaluated the genetic differences and structuring along the species distribution. Here we used both microsatellite (10 loci) and mtDNA markers to evaluate the population structure and evolutionary history of the species. We found significant structuring between Pacific and Atlantic populations that corresponds to two reciprocally monophyletic mitochondrial lineages separated since early Pleistocene, indicating extreme female phylopatry. We also found significant genetic structure between intra-oceanic breeding sites. Microsatellites analyses also found the populations from the two oceans as significantly different with several private alleles, although very small inter-oceanic gene flow mediate by males could not be discarded. Our results show that the species did not suffer recently any significant reduction of its genetic diversity. Our findings strongly support that *O. flavescens* Atlantic and Pacific populations are two evolutionary significant units (ESUs) and that intra-oceanic breeding colonies should also be managed separately.

INTRODUCTION

The Southern sea lion, *Otaria flavescens* Shaw 1800, belongs to the Otariidae or eared seals, which is comprised by Fur Seals and Sea Lions. Despite the limited fossil record, recent evolutionary biogeographic studies corroborated previous ideas about otariids eastern North Pacific origin, at middle Miocene (Déméré et al. 2003; Arnason et al. 2006). The earliest known otariid fossil is *Pithanotaria starri*, found in the Sisquoc Formation of California, USA, from Late Miocene. It is suggested that the otariids dispersal to southern oceans in a single or a few events of equator crossing from North to South Pacific via eastern boundary currents, with a posterior colonization of the Atlantic side of South America (Déméré et al. 2003; Arnason et al. 2006). Nevertheless the dates of those events are largely unknown.

Otaria flavescens is widely distributed along the South American coast (Fig. 1), from southern Brazil to Cape Horn, including Falkland Islands, in the Atlantic Ocean; and from Cape Horn to Peru in the Pacific Ocean (Bastida et al. 2007; Capozzo 2002;). Besides these reproductive sites, animals are also found sometimes northern of their normal range in both oceans (Bastida et al. 2007).

The Southern sea lion is the biggest otariid of its region. The males weigh up to 350 kg and measure around 3 m in length and the females weight up to 150 kg and measure about 2.2 m (Capozzo 2002; Bastida et al. 2007). Like all eared seals, it is gregarious and polygamous (Hamilton 1934), and from December to March, breeding colonies formed by harem bulls, pregnant cows and newly born pups can be found at several islands as well as in mainland (Capozzo 2002; Bastida et al. 2007). The males come first to guarantee territory and wait for females. The number of females per harem depends on the size of the male's territory, varying

from 4 to 9 cows for each bull. It cannot be exactly stated when the breeding begins or ends (Hamilton 1934), and the peak of reproduction can vary among colonies. As the end of the summer approaches, they become more spread and solitary. *Otaria flavescens* does not perform mass migrations, although, they can disperse significantly in the water and make seasonal movements (Vaz- Ferreira 1965).

For the ancient people of southern South America this species was a very important source of food and heat. They were commonly hunted because of its meat, skin and specially blubber (Bastida et al. 2007; Capozzo 2002). In the last two centuries, the exploration of this resource was an important economic activity in Uruguay, until the prohibition of hunting, at the beginning of the ninety decade. However, there are some reports of illegal hunt and of death resulting of fishing interactions. Currently, this is the main problem concerning *O. flavescens* conservation. (Ott et al. 1996; Capozzo 2002; Crespo & Dans 2005). Recent studies about population size are rare, but around 250,000 specimens are believed to exist in all distribution range (Seal Conservation Society, www.pinnipeds.org).

Despite its wide distribution and interactions with humans, few studies were carried out on their evolutionary history, genetic diversity and population differentiation. Szapkievich et al. (1999) used protein electrophoresis to estimate genetic distance between two rookeries in the Atlantic coast, one from Patagonia, Argentina and the other from Uruguay. They did not find evidence of structure and these two rookeries seemed to belong to the same population. On the other hand, Brunner (2004), performing traditional morphometry of 55 skulls from Atlantic side mainland and Falkland Islands, found significant differences which separated the specimens into two groups, corresponding to their collection site.

90 Recently, two studies compared the Pacific and Atlantic specimens. Drehmer (2005)
91 measured skulls, using traditional and geometric morphometry. He found four geographic groups
92 with distinct characteristics, two in each side of the continent, but no systematic or management
93 proposal was suggested. In 2007, Túnez et al. were the first to use mitochondrial DNA (mtDNA)
94 sequencing to compare *O. flavescens* populations from two regions of Atlantic coast and the
95 Peruvian coast. They sequenced a fragment of the cytocrome b and found six haplotypes. No
96 haplotypes were shared between the populations of the two sides of the continent. Given this
97 results they proposed two different evolutionary significant units, one in Atlantic and other in
98 Pacific. Nevertheless, only five sequences from Peru were used to compare the population from
99 each ocean and no nuclear markers were analyzed in the study.
100 The evolutionary significant unit (ESU) concept has been extensively debated since the
101 term was coined by Ryder (1986). In the *O. flavescens* case, the use of only mitochondrial DNA
102 (mtDNA) as a molecular marker to define an ESU should be viewed with caution. Given the
103 exclusively maternal transmission of the mtDNA, it only reports the evolutionary history of
104 females (Avise 1987). Given that a phylopatric behavior has been described for the females of the
105 Southern sea lion (Riedman 1990; Fabiani et al. 2003) but not for males, population structuring
106 found through mtDNA analysis exclusively could give a partial picture of the gene flow between
107 the populations, being necessary the use of biparental markers to have a more complete
108 evaluation of the species population structure. However, no such study was presented so far.
109 We present here the first study of *O. flavescens* comprising two regions of mtDNA
110 (control region and cytochrome b) and ten loci of microsatellites with a broader sampling of both
111 oceans. Our aim is to answer the following main questions: Did the difference between the

Pacific and Atlantic populations maintain when much more Pacific, including Chilean, samples are added? Can we find population structuring in nuclear DNA markers? Which is the level of this structuring? Is it in agreement with mtDNA? How old are the divergence in mtDNA? Did the recent depopulation cause by commercial hunting significantly affect the genetic diversity of the populations?

MATERIAL AND METHODS

Sample collection and DNA extraction

A total of 76 *O. flavescens* tissues samples were collected: 29 from the hind flippers of live pups in Punta San Juan, Peru; nine in Isla Guafo and two in Isla Chañaral, Chile; 27 from death animals found stranded ashore in Argentina; and 17 from southern Brazilian Coast (Fig. 1). The samples were stored in ethanol 70% or DMSO. Genomic DNA extractions were performed with standard phenol-chloroform (Sambrook et al. 2001) and NaCl protocols (Medrano et al. 1990) or the DNeasy Tissue Kit (Qiagen). Since most of the samples from Argentina were too degraded to be genotyped, they were excluded from the nuclear microsatellites analyses. However, we were able to obtain mtDNA sequence information from 19 of those 27 samples.

Mitochondrial DNA amplification and analysis

We amplified part of the mtDNA control region and cytochrome b (cyb) gene by PCR using the following primers: R3(L15926)THR 5′- TCA AAG CTT ACA CCA GTC TTG TAA ACC - 3′ (Kocher et al. 1989); TDKD(H16498) 5′- CCT GAA GTA GGA ACC AGA TG - 3′ (Meyer et al. 1990) for the control region; and GLUDG-L and CB2-H (Palumbi & Kessing 1991)

for cyb. Amplifications were carried out in 20µl with the following conditions: 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.1 µM of each primer, 1 U of Platinum *Taq* DNA polymerase (Invitrogen), 1X PCR buffer (Invitrogen), 0.2% - 0.4% Triton and 2 µl of DNA (approximately 50 ng)**.** Thermocycling conditions for control region amplification were: 3min at 94 ºC, and 10 cycles of "touchdown", each including 50 s at 94 °C, 50s at 60 °C (-1º/cycle), and 80s at 72 °C; 30 cycles of 50s at 94 °C, 50s at 50 °C and 80s at 72 °C; followed by a final extension of 5min at 72 °C. The parameters used in the amplification of the cyb were the same of the control region without the first 10 cycles of "touchdown". Amplification products were purified with shrimp alkaline phosphatase and exonuclease I (Amersham Biosciences). The purified were sequenced in both directions using the DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences) and run in a MegaBace 1000 automated sequencer (Amersham Biosciences).

The chromatograms were checked by eye in FinchTV v.1.4 (www.geospiza.com/finchtv.html) and a consensus sequence of each individual was generated using Phred-Phrap (Ewing et al. 1998). Each consensus sequence was rechecked and edited in BioEdit 7.0.5 (Hall 1999) if necessary. Alignments were performed using ClustalW (Thompson et al. 1994) and corrected by eye. The program Network v.4.5 (www.fluxus-engineering.com) was used to generate a median-joining haplotype network (Bandelt et al. 1999) of the cyb, control region, and concatenated sequences (as specified in Table 1). The network of the cyb sequences was rooted with a sequence of *Zalophus californianus*. Networks with the control region were not rooted due the large differences found between the studied species and the outgroup.

The *O. flavescens* cyb sequences were aligned (360 bp) with sequence of other otariids downloaded from Genbank (Table 1) to estimate the times of the most recent common ancestor

156 (TMRCAs) using the uncorrelated lognormal relaxed molecular clock Bayesian approach
157 developed in the software BEAST 1.4.8 (Drummont et al. 2006; Drummond & Rambaut 2007).
158 To calibrate the molecular clock we used the confidence interval of the divergence between
159 *Arctocephalus fosteri* and *Zalophus californianus* (1.7 Ma [million years ago] – 6.3 Ma, E.
160 Eizirik, personal communication) as an uniform prior. We used the HKY substitution model with
161 gamma site heterogeneity with eight categories. A chain with 50,000,000 steps was run, taking
162 into account the stabilization of the traces and the parameters sampling.
163 Population size changes were estimated using the Bayesian Skyline plot method developed
164 in BEAST to the control region dataset. This method works through the estimate of the effective
165 population size (Ne) through time (Drummond et al. 2005). We used a GTR and gamma +
166 invariant sites model with a relaxed molecular clock as above with a mean rate of 0.0368/site/Ma
167 from Tchaika et al. (2007). These analyses were run separately for the Pacific and Atlantic
168 populations (see below).
169 Basic statistics and analysis of molecular variance (AMOVA, Excoffier et al. 1992) and F-
170 statistics (F_{ST}; Hudson et al. 1992) was carried out in Arlequin v.3.11 (Excoffier et al. 2005).
171
172 *Microsatellite amplification and analysis*
173 Since no specific microsatellite loci were developed so far for *O. flavescens*, we first
174 evaluated a large number of loci previously developed for other pinnipeds. We finally chose ten
175 loci of dinucleotide short tandem repeats (STR): ZcwB07, ZcwE04, ZcwG04, ZcwF07 and
176 ZcwE12, developed for *Z. californianus*; Hg8.10 and Hg6.3, developed for *Halichoerus grypus*;
177 Pvce and Pv9 described for *Phoca vitulina*; and M11 described for *Mirounga* sp. (Allen et al.

178 1995; Coltman et al. 1996; Gemmel et al. 1997; Hoffman et al. 2007). Forward primers were 5'-
179 tailed with the M13 sequence (5'-CACGACGTTGTAAAACGAC-3') that was used in
180 combination with a M13 primer marked with fluorescence (FAM, HEX, NED) (Boutin-Ganache
181 et al. 2001). Amplifications were carried out in 10 µL with the following conditions: 1.5 mM
182 $MgCl_2$, 200 µM of each dNTP, 0.5 mM of reverse and M13-fluorescent primers, 0.0333 mM of
183 the M13-tailed forward primer, 0.5 U of Platinum *Taq* DNA polymerase (Invitrogen), 1X PCR
184 buffer (Invitrogen), 0.6% of Trehalose and 1 µl of DNA (approximately 50 ng). Thermocycling
185 conditions for the amplification of the loci ZcwB07, ZcwE04, ZcwG04, ZcwF07, ZcwE12,
186 Hg8.10, M11 and Pvce were the same of the mitochondrial control region (see above). For the
187 Pv9 and Hg6.3 loci the conditions were: 2 min at 94 °C; 25 cycles of 45 s at 94 °C, 45s at 58 °C,
188 50 s at 72 °C; and a final extension of 2 min at 72 °C. The PCR products were genotyped on a
189 MegaBACE 1000 automated sequencer (Amersham Biosciences). The allele size in number of
190 bases was identified with the software Genetic Profiler version 2.2 (Amersham Biosciences).
191 To plot histograms of the allele frequencies, serch for private alleles and perform a pairwise
192 principal components analysis (PCA) to population assignment we used the program Genalex.6
193 (Peakal and Smouse 2006). We tested for deviations from Hardy-Weinberg equilibrium (HWE)
194 and linkage disequilibrium (LD) using the program Arlequin 3.11. Significance levels ($\alpha = 0.05$)
195 for departure from HWE and for LD were corrected for simultaneous comparisons with the
196 sequential Bonferroni test (Rice 1989). Expected heterozygosity (He), observed heterozygosity
197 (Ho), *F*-statistics (F_{ST} and ϕ_{ST}) and AMOVA analysis were calculated using Arlequin.
198 To evaluate the genetic structure of the species we also used the Bayesian approach
199 implemented in the program Structure v.2.2 (Pritchard et al. 2000). We tested the number of

clusters (*K*) using the calculations suggested by Pritchard et al. (2000) and Evanno et al. (2005). We set from 1 *K* to 6 *K*, carrying out 10 independent runs with 100,000 of burn-in and 100,000 replications for each *K*. This burn-in length was used taking into account the stabilization of posterior probability. We ran the Markov Chain twice, assuming the admixture model with correlated and independent allele frequencies. To identify possible migrants, or an individual that has ancestors from different clusters, we tested the same set of data switching the admixture model to population information. Another similar analysis was carried out in the program Structurama (Huelsenbeck and Andolfatto 2007). The program uses a particularly efficient variant of MCMC called Gibbs sampling, where each MCMC cycle involves a Gibbs scan of all of the individuals. Hence the total number of MCMC cycles for the analysis of this study is the product of the reported number of MCMC cycles and the number of individuals in the analysis. In this way, to infer the number of clusters and the population genetic structure, we set number of populations to be a random variable, a parameter that uses a Dirichlet process prior (Pella and Masuda 2006). We ran 1,000,000 cycles, with gamma distribution (θ=2, k=3) for the random variable prior of the number of populations. The first 100,000 cycles were discarded as burn-in.

RESULTS

Mitochondrial DNA

We sequenced and aligned 67 samples for the control region (420 bp) and 63 samples for cyb (402 bp), which resulted in 37 haplotypes and 40 polymorphic sites for control region, and 9 haplotypes and 15 polymorphic sites for cyb. As expected given it is non-coding, control region shows higher levels of haplotype (H_d) and nucleotide diversity (π) comparing to cyb (Table 2).

222 For the control region, the four sampling regions, as well as the Pacific and Atlantic samples have
223 similar values of high haplotype and relatively low (~0.8%) nucleotide diversity. On the other
224 hand, the whole species has a high genetic diversity of ~2%.

225 The three mtDNA haplotype networks show two major and highly divergent clades
226 corresponding perfectly to individuals that inhabit each ocean (Figure 2), demonstrating a high
227 inter-oceanic genetic structure. The cyb network, although less informative for intra-oceanic
228 samples, presents seven mutational steps between the two ocean clades (Figure 2C). The position
229 of insertion of the outgroup node, *Z. californianus*, at about the middle of the branch separating
230 the two ocean clades suggests an ancient divergence between these clades. With the use of the
231 more variable control region (Figure 2A,B) there is no shared haplotypes between the four
232 sampling regions, the networks showing a clear signal of intra-oceanic geographic structure .

233 The AMOVA analyses show that most of the variance is among ocean groups (Table 3) All
234 the pairwise Fst and Φst values between sampling localities are high and significant, with
235 exception of the comparison between Brazil and Argentina for the cyb, being generally much
236 higher (especially for the Φst values) between areas that are located on different oceans (Table
237 4).

238 The estimated TMRCA of all *O. flavescens* cyb haplotypes, that dates the divergence of the
239 two oceanic clades, goes back to the early Pleistocene, with a mean of 1.78 Ma and a 95%
240 confidence interval from 3.8 Ma to 0.4 Ma. The TMRCA of the Atlantic and Pacific clades were
241 very similar, with a mean around one Ma and the 95% confidence interval between 0.15-2.3 Ma.
242 The mean substitution rate for the cyb fragment estimated for the otariid by BEAST in this
243 analysis was 1.6% /site/Ma, that is very similar to the mean rate described in the literature

244 (Moritz and Hillis 1990). Bayesian Skyline plots from the control region datasets show a weak
245 signal of expansion of the Pacific population since about 100,000 years ago, and no demographic
246 change in the Atlantic population (Figure 3).
247
248 *Microsatellites*
249 We genotyped 53 samples from Peru (29), Chile (9) and Brazil (15) (see Table 1 for
250 details) for 10 microsatellite loci, with only 3% of missing data. With Bonferroni correction, two
251 loci were in linkage disequilibrium: ZcwG04 with ZcwE04 only for the Brazilian sample; and
252 three deviated from Hardy-Weinberg equilibrium (Table 5).
253 Both analyses of the number of population clusters suggest the existence of two
254 populations. The number of *K=2* had the best probability estimated by Structure 2.2 in both
255 models, correlated and independent allele frequencies (Pritchard et al. 2000; Evanno et al. 2005).
256 Structurama also estimated *K*=2 as having the best probability (P (*K*/2) = 0.9620). All individuals
257 from Peru and Chile were assigned to belong to cluster 1, while all individuals from Brazil were
258 assigned to belong to cluster 2 by both programs (Figure 4A). Pairwise PCA indicated the
259 existence of two groups comprising individuals from Chile and Peru in one group and other
260 formed by Brazilian specimens (Figure 4B). Only one individual from Chile (C3) was assigned
261 by Structure as having almost equal probabilities to belong to both clusters. When we reran the
262 analysis switching admixture model to population information this individual was recognized as
263 migrant or descendent of both clusters. This structure based on microsatellite completely agrees
264 with the two major mitochondrial lineages. Private alleles are present in all loci for these two
265 clusters, and in some loci the difference in allele frequencies are evident (Figure 5). AMOVA and

pairwise Fst and Rst were usually significant and higher between populations from different oceans (Table 3 and 6).

DISCUSSION

Phylogeographical pattern and population structure

Our mitochondrial results indicate an ancient evolutionary history for the Southern sea ion in South America, with an old (~1.7 Ma) divergence between Pacific and Atlantic populations, which suggests the existence of a long term effective barrier to female migration or a historical event that leaded to a separation between two major oceanic clades. Moreover, the Pacific populations seem to be less structured than the Atlantic populations. The microsatellite analyses agree with the mitochondrial results, presenting significant structure between Atlantic and Pacific populations and a weak structure between the two Pacific populations. This situation is similar to that of the Steller's sea lion, which was found concordance between major mtDNA and STR structuring in a somewhat continuous distribution (see Hoffman et al. 2006). Recently, Oliveira et al. (2008) also found significant differences in allele frequencies in seven microsatellite loci between Peruvian and Uruguayan Southern fur seals (*Arctocephalus australis*). Therefore, there is a clear barrier to gene flow between Atlantic from Pacific populations of *O. flavescens*, apparently absolute for the females, that is likely located around the southern tip of South America. To locate more precisely the genetic break between the populations would need more exhaustive sampling in this region. The nature of this long standing breakup is not known, but it is likely a combination of ecological barriers with the high female phylopatry of the pinnipeds.

288 *Female phylopatry and male biased gene flow*

289 Female natal site fidelity is widely described in pinnipeds and has been demonstrated by
290 molecular techniques in several species, like *Eumetopias jubatus* (Steller's sea lion)*, Mirounga*
291 *leonina* (elephant seal) and *Neophoca cinera* (Australian sea lion) (Bickham et al. 1996; Slade et
292 al. 1998; Hoffman et al. 2006; Fabiani et al. 2006; Campbel et al. 2008). This behavior could in
293 extreme situations lead to the reciprocal monophyly of mitochondrial haplotypes. Our results
294 point that the Atlantic and Pacific populations of the Southern sea lion were devoid of female
295 gene flow in the last around one million years, suggesting that female phylopatry seems very
296 strong in this species. The absence of shared haplotypes between the main sampling locations
297 corroborates this hypothesis. One possible evolutionary reason that could favor female
298 phylopatry is the reproductive success generated by gregarious mating systems, which promote
299 easy encounter and reduce predation risk, in addition to the increasing of fitness promoted by
300 interaction with relatives and cooperative breeding (Hamilton 1964; Riedman 1990). For otariids,
301 diminution of male harassment by the increasing of female aggregation has been proposed as an
302 important factor for the augmentation of the female assembly (Cassini 2000; Capozzo 2008).
303 Recently, Grandi et al. (2008) studying the social distribution of colonies and how new breeding
304 arise suggested that the new breeding colonies are not established at random, but near colonies
305 where conspecifics breed, another indication of phylopatry. These trends raise difficulties in
306 colonization of distant areas and in female gene flow among breeding colonies, promoting
307 separation of the female stocks.

308 In several mammals and mainly in pinnipeds there are indications of male biased gene
309 flow, a trait of highly polygynous mammals (Greenwood 1980; Dobson 1982; Fabiani et al.

2003). In polygynous mammals, young males are more prone to disperse (Dobson 1982). In *O. flavescens*, young males are usually outside from the center of breeding areas and tend to stand in the periphery (Grandi et al. 2008; Capozzo et al. 2008). Their reproductive strategy consists to harass females away from an adult domain male (Trillmich & Trillmich 1984). Without a domain, young males and adults that could not get harems are free to disperse and visit neighbor colonies, increasing their chances of female harassment and territory establishing. This likely lead to some male gene flow between neighbor colonies. Another possibility is that males disperse for long distances among breeding colonies helped by marine currents. This possibility was already pointed out for two pinniped species. Using molecular methods, Fabiani et al. (2003) identified a possible male of Southern elephant seal that can have traveled to breed in a colony 8,000 km away from its birth site. Ferreira et al. (2008) also used molecular markers to identified putative vagrant males of Amsterdam fur seals (*Arctocephalus tropicalis*) found in Brazilian coast. Their results show that these males came from different breeding areas, an evidence of male dispersion for long distances. *Otaria flavescens* has a costal foraging behavior, feeding of several species and performing flatter dives (Bastida et al. 2007). This makes neighboring dispersion more likely in this species in absence of genetic barriers. These agree well with our results for the microsatellites, despite our limited sampling sites, since the Fst divergence between the Chilean and Peruvian populations were very small and between these two and the Atlantic population were high and significant, although the distance between the Atlantic populations and the Chilean is not much greater than between the latter and the Peruvian. However, Chilean C3 sample seems to be of mixed Atlantic-Pacific ancestry, suggesting rare inter-oceanic male gene flow may also occur.

332

333 *Demography*

334 Our results indicate the existence of large historical population sizes of Southern sea lion
335 from both Atlantic and Pacific oceans. Therefore, despite the population decreasing caused by
336 commercial and illegal hunt, our results show that none of these events were able to reduce
337 significantly the effective population size and reduce genetic diversity of that populations. Only a
338 very drastic reduction of the effective population size during some generations or a not so drastic
339 reduction during several generations can decrease significantly the genetic diversity of one
340 species (Frankham et al. 2002). The high female phylopatry and the consequent genetic
341 structuring may have contributed significantly to the maintenance of the overall high diversity of
342 these two oceanic regions. Interestingly, both the Atlantic and Pacific populations show very
343 similar TMRCAs (~ 1 Ma) and effective population sizes for the mtDNA data, as seen by the
344 Bayesian skyline plots (Fig. 3). The weak expansion around 100,000 years ago from a little
345 smaller size found for the Pacific population may be related to the severe climatic changes in the
346 Pacific coast during most of the Pleistocene. It has been reported that the El Niño Southern
347 Oscillation (ENSO) cause reduction of pinnipeds population in Peruvian region throughout
348 changes the sea water temperature, reducing the food availability (Majluf 1998). The several
349 successive ENSOs during the evolutionary history of the species could have cause a reduction-
350 expansion population dynamic that shaped the mitochondrial pattern of the Pacific, in special the
351 Peruvian population.

352

353 *Conservation implications*

354 Our results suggest that the Atlantic and Pacific populations should be treated as different
355 evolutionary significant units (ESU), based on the concordance between the mtDNA and nuclear
356 DNA datasets, indicating the inexistence of female migrations between the oceans and male
357 migration may occur but is a rare event. The extinction of the Southern sea lion in Atlantic or
358 Pacific coasts would imply a loss of half the genetic diversity of the species and a very ancient
359 mitochondrial clade. Due the high female phylopatry detected here, which would highly difficult
360 colonization of new areas, each intra-oceanic breeding colony should also be managed separately,
361 specially in the Atlantic coast, where a stronger structuring is evident and the breeding colonies
362 are better defined (Túnez et al. 2007; 2008). This also implies the necessity of more studies and a
363 better definition of the Pacific breeding colonies.

364

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519

FIGURE LEGENDS

Figure 1: Distribution of the Southern sea lion and sampling localities.

Figure 2: Haplotype networks of mtDNA sequences. The circles are proportional to the frequency of the haplotypes. Branches are proportional to the number of mutational steps. The lines indicate the number of mutational steps of branches that present more than one mutation. (A) haplotype network of the concatenated sequences (822bp). (B) haplotype network of the control region fragment (420bp). (C) haplotype network of the cyb fragment (402bp).

Figure 3: Bayesian skyline plot showing the effective population size fluctuation throughout time. Recent time is on the left side (black line is the median estimation; gray line is the confidence interval). (A) Pacific, (B) Atlantic

Figure 4: (A) Structure bar plot. Each bar is one individual and each color represents the probability of the individual to belong to determinate cluster, the arrow shows the individual identified as a possible migrant or a descendent of both clusters. (B) Pairwise population assignment through PCA among sampling localities.

Figure 5: Allele frequencies for each locus in each cluster. Yellow bars represent the frequencies in Atlantic cluster, green bars represent the frequencies in Pacific cluster.

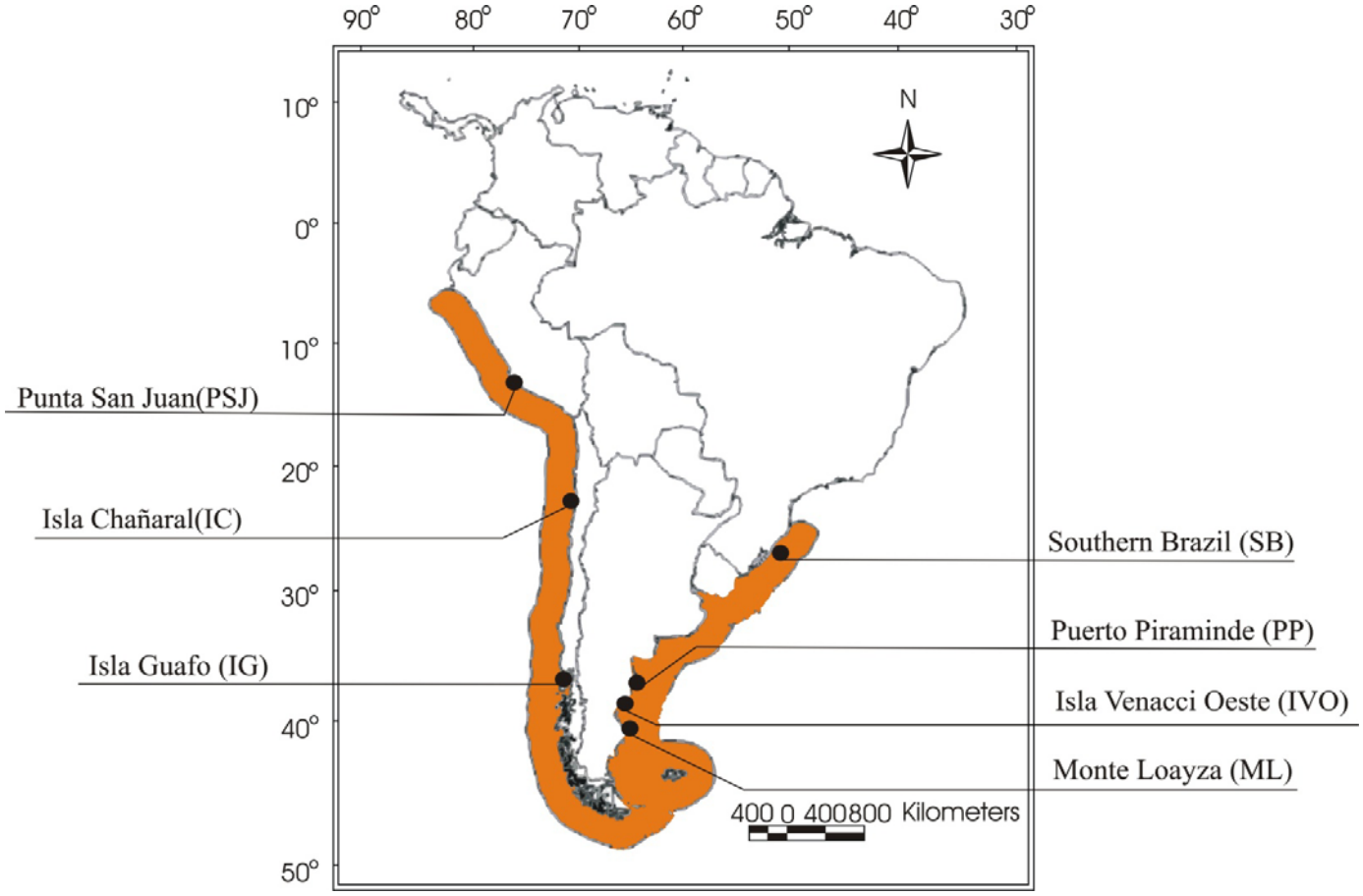
90°
80°
70°
60°
50°
40°
30°
10°
0°
10°
20°
30°
40°
50°
N
Punta San Juan(PSJ)
Isla Chañaral(IC)
Isla Guafo (IG)
Southern Brazil (SB)
Puerto Piraminde (PP)
Isla Venacci Oeste (IVO)
Monte Loayza (ML)
400 0 400800 Kilometers

Figure 1

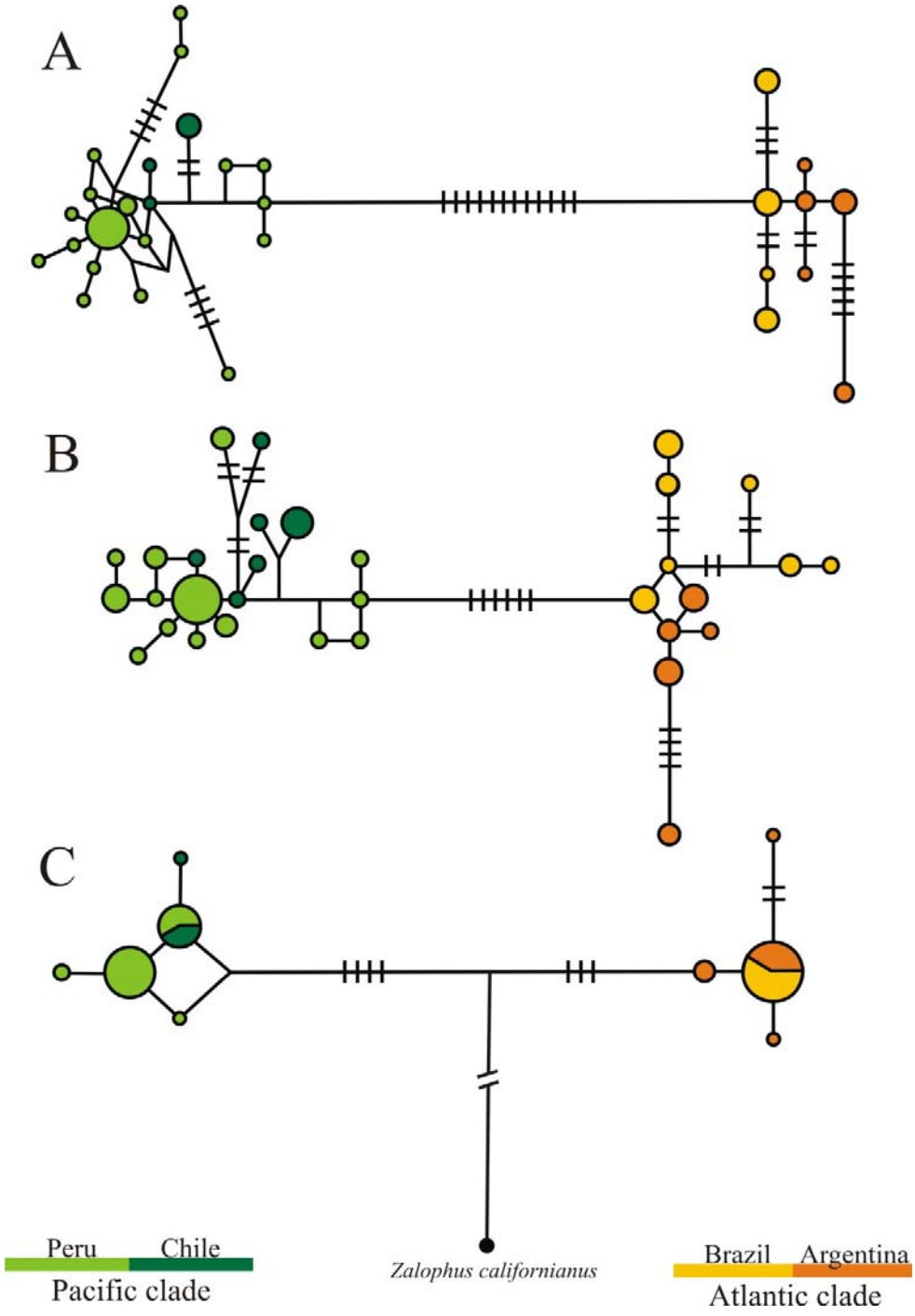
A
B
C
Peru
Chile
Pacific clade
Zalophus californianus
Brazil
Argentina
Atlantic clade

Figure 2

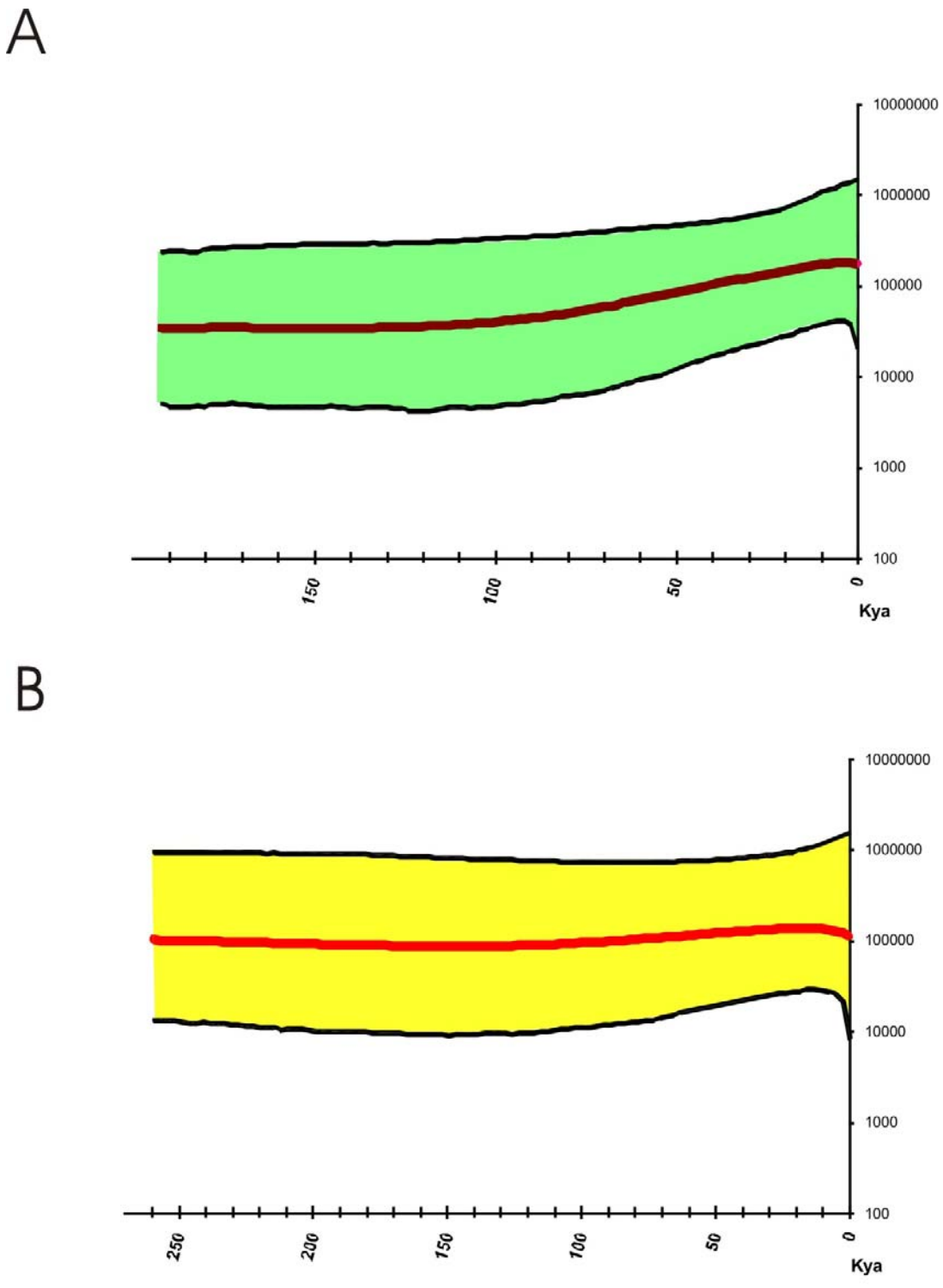
A
10000000
1000000
100000
10000
1000
100
150
100
50
0
Kya
B
10000000
1000000
100000
10000
1000
100
250
200
150
100
50
0
Kya

Figure 3

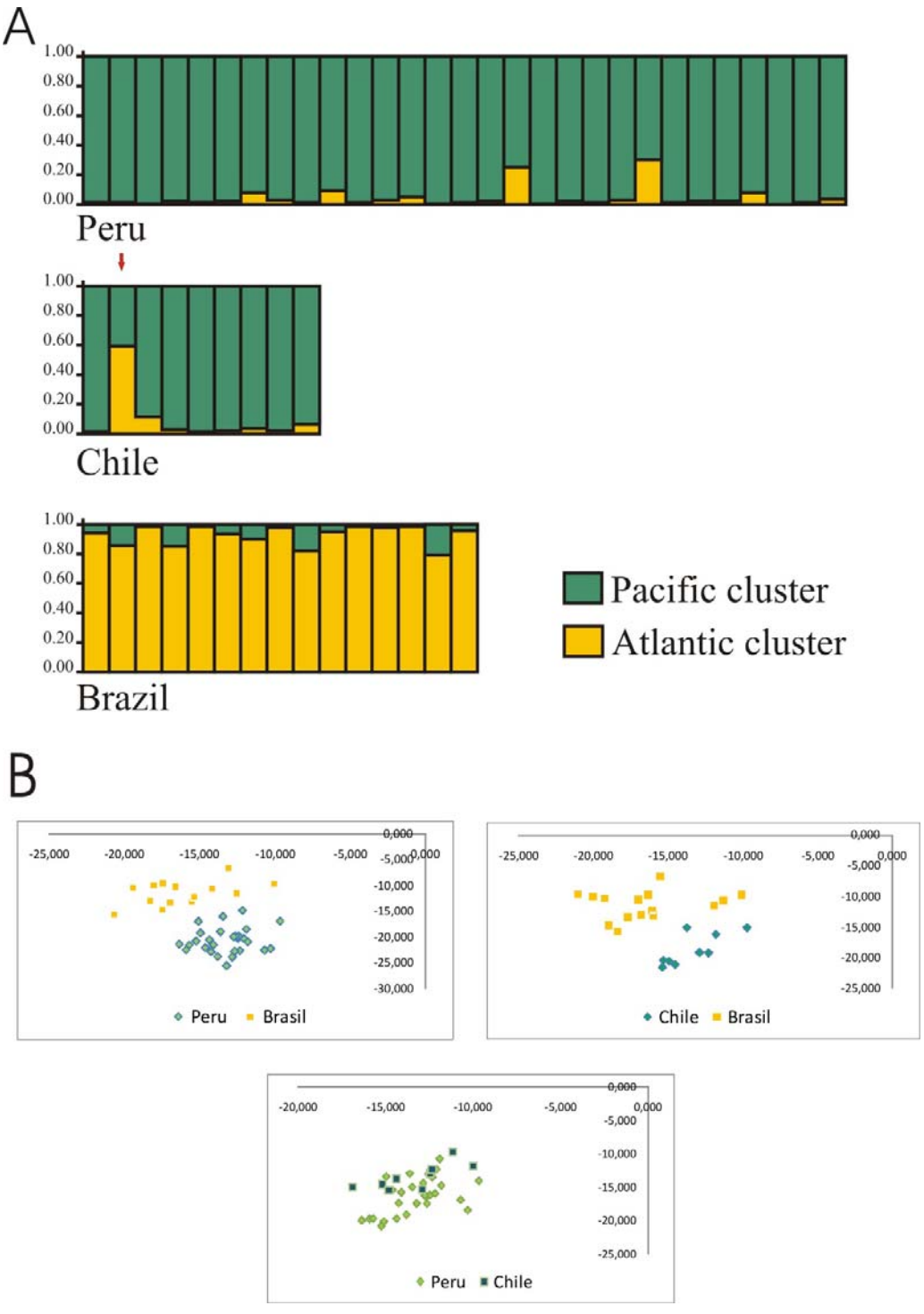
A
1.00
0.80
0.60
0.40
0.20
0.00
Peru
Chile
Brazil
Pacific cluster
Atlantic cluster
B
Peru
Brasil
Chile
Brasil
Peru
Chile

Figure 4

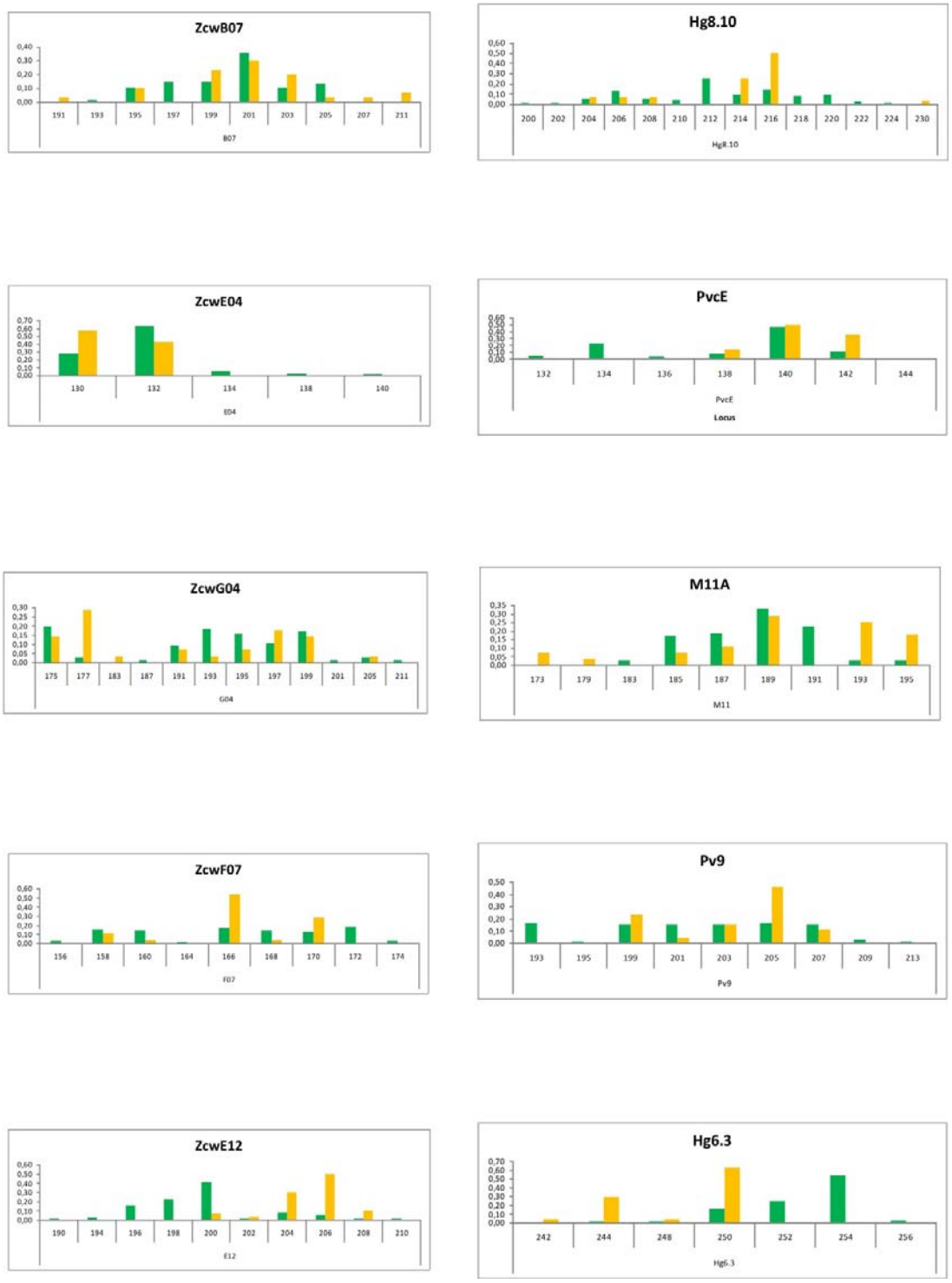
ZcwB07
B07
Hg8.10
HgR.10
ZcwE04
E04
PvcE
PvcE
Locus
ZcwG04
G04
M11A
M11
ZcwF07
F07
Pv9
Pv9
ZcwE12
E12
Hg6.3
Hg6.3

Figure 5

Table 1: List List of samples with the respective locality, molecular marker used and GenBank accession numbers (GenBank would be completed after the submission of the sequences generated here to the NCBI).

Sample	locality	DNA marker used		
		Cyt-b	Control Region	STR
P01	Punta San Juan	x	x	x
P02	Punta San Juan	x	x	x
P03	Punta San Juan	x	x	x
P04	Punta San Juan	x	x	x
P05	Punta San Juan	x	x	x
P06	Punta San Juan	x	x	x
P07	Punta San Juan	x	x	x
P08	Punta San Juan	x	x	x
P09	Punta San Juan	x	x	x
P10	Punta San Juan	x	x	x
P11	Punta San Juan	x	x	x
P13	Punta San Juan	x	x	x
P14	Punta San Juan	x	x	x
P15	Punta San Juan	x	x	x
P16	Punta San Juan	x	x	x
P17	Punta San Juan	x	x	x
P18	Punta San Juan	x	x	x
P19	Punta San Juan	x	x	x
P20	Punta San Juan	x	x	x
P21	Punta San Juan	x	x	x
P22	Punta San Juan	x	x	x
P23	Punta San Juan	x	x	x
P24	Punta San Juan	x	x	x
P25	Punta San Juan	x	x	x
P26	Punta San Juan	x	x	x
P27	Punta San Juan	x	x	x
P28	Punta San Juan		x	x
P29	Punta San Juan	x	x	x
P30	Punta San Juan	x	x	x
NC15	Isla Chañaral		x	
NC28	Isla Chañaral		x	
C1	Isla Guafo		x	x
C3	Isla Guafo		x	x
C4	Isla Guafo	x	x	x
C5	Isla Guafo	x	x	x
C6	Isla Guafo	x	x	x
C7	Isla Guafo	x	x	x
C8	Isla Guafo	x	x	x
C9	Isla Guafo			x
C10	Isla Guafo	x	x	x
IVO1	Isla Venacci Oeste		x	
IVO2	Isla Venacci Oeste	x	x	
IVO3	Isla Venacci Oeste	x	x	
ICO4	Isla Venacci Oeste		x	

Sample	locality	DNA marker used		
		Cyt-b	Control Region	STR
IVO5	Isla Venacci Oeste		x	
IVO6	Isla Venacci Oeste	x		
IVO7	Isla Venacci Oeste	x	x	
IVO8	Isla Venacci Oeste	x		
IVO10	Isla Venacci Oeste	x	x	
ML1	Monte Loayza	x		
ML2	Monte Loayza	x	x	
ML3	Monte Loayza		x	
ML6	Monte Loayza	x		
ML7	Monte Loayza	x	x	
ML8	Monte Loayza	x		
ML10	Monte Loayza	x		
PP5	Puerto Piramide	x	x	
PP6	Puerto Piramide	x	x	
PP7	Puerto Piramide	x	x	
G516	Southern Brazil		x	x
G517	Southern Brazil	x		x
G553	Southern Brazil	x	x	x
G554	Southern Brazil	x	x	x
G555	Southern Brazil	x		x
G658	Southern Brazil		x	x
G667	Southern Brazil	x	x	
G809	Southern Brazil	x	x	x
G812	Southern Brazil	x	x	x
G813	Southern Brazil	x	x	x
G822	Southern Brazil		x	x
G868	Southern Brazil	x		x
G967	Southern Brazil	x	x	x
G992	Southern Brazil	x	x	x
G1178	Southern Brazil	x	x	x
G1189	Southern Brazil	x	x	x
Gordo	Southern Brazil	x	x	
Zalophus californianus		AM422163.1		
Eumetopias jubatus		DQ145021.1		
Neophoca cinerea		AF380913		
Phocarctos hookeri		AF380919		
Arctocephalus australis		AY712974.1		
Arctocephalus forsteri		X82293.1		
Arctocephalus gazella		X82292.1		
Arctocephalus philippii		AF380893		
Arctocephalus pusillus doriferus		AF380918		
Arctocephalus pusillus pusillus		APU18454		
Arctocephalus townsendi		AF380897		
Arctocephalus tropicalis		AF380883		

Table 2: Genetic diversity of each locality for the different mitochondrial regions. (N) number of individuals analyzed, (*h*)number of haplotypes, (H_d) haplotype diversity, (π)nucleotide diversity.

	Cyt-b				Control region				Concatenated			
	N	*h*	Hd	π	N	*h*	Hd	π	N	*h*	Hd	π
Atlantic	29	4	0.3128 +/-0.1062	0.000993 +/-0.001057	27	15	0.9544 +/-0.0185	0.009669 +/-0.005536	20	9	0.9105 +/-0.0323	0.004804 +/-0.002808
Brazil	13	1	0	0	14	8	0.9121 +/-0.0486	0.008914 +/-0.005353	11	4	0.7818 +/-0.0749	0.003350 +/-0.002164
Argentina	16	4	0.5167 +/-0.1324	0.001741 +/-0.001552	13	7	0.8974 +/-0.0537	0.005725 +/-0.003732	9	5	0.8611 +/-0.0872	0.004566 +/-0.002890
Pacific	34	5	0.6078 +/-0.0568	0.001787 +/-0.001525	38	22	0.9317 +/-0.0290	0.008301 +/-0.004789	33	21	0.9072 +/-0.0441	0.005593 +/-0.003128
Chile	6	2	0.3333 +/-0.2152	0.000829 +/-0.001092	10	7	0.8667 +/-0.1072	0.007511 +/-0.004783	5	3	0.7000 +/-0.2184	0.005048 +/-0.003518
Peru	28	4	0.5370 +/-0.0862	0.001487 +/-0.001362	28	15	0.8889 +/-0.0497	0.007329 +/-0.004354	28	18	0.8783 +/-0.0595	0.004948 +/-0.002827
Overal	63	9	0.7445 +/-0.0314	0.012872 +/-0.007004	65	37	0.9692 +/-0.0108	0.019090 +/-0.009941	53	30	0.9521 +/-0.0181	0.016102 +/-0.008159

Table 3: AMOVA analyses for each sequence fragment and the microsatellites, each ocean corresponds to one group.

Source of variation	Percentage of variation				
	ϕst-parewise differences			FST	RST
	Control region	Cyt-b	Concatenated	STR	
Among groups	64.18	92.66	74.95	8.81	6.43
Among populations within groups	11.03	2.39	8.77	1.70	8.18
Within populations	11.03	4.95	16.28	89.49	85.39

Table 4: Pairwise *F*-statistics among sampling localities and between Atlantic and Pacific oceans for each mitochondrial fragment. *$P < 0,05$

		Cyt-b				Control region				Concatenated			
		Brazil	Arg	Chile	Pacific	Brazil	Arg	Chile	Pacific	Brazil	Arg	Chile	Pacific
Argentina	Fst	0.15				0.09*				0.18*			
	ϕst	0.05				0.37*				0.40*			
Chile	Fst	0.90*	0.54*			0.11*	0.12*			0.25*	0.20*		
	ϕst	0.99*	0.93*			0.74*	0.79*			0.86*	0.84*		
Peru	Fst	0.66*	0.47*	0.40*		0.10*	0.11*	0.12*		0.16*	0.12*	0.18*	
	ϕst	0.96*	0.93*	0.49*		0.73*	0.77*	0.24*		0.83*	0.83*	0.32*	
Atlantic	Fst				0.53*				0.06*				0.09*
	ϕst				0.94*				0.70*				0.80*

Table 5: Genetic diversity of each locus per locality, per cluster and overall. (A) number of alleles, Ho observed heterozygozity, (He) expected heterozygozity.* Loci that deviated from H-W equilibrium after Bonferroni correction.

Locus	Peru			Chile			Brazil			Pacific			Atlantic			Overall		
	A	Ho	He	A	Ho	He	A	Ho	He	A	Ho	He	A	Ho	He	A	Ho	He
ZcwB07	6	0,83	0,8	6	0,55	0,73	8	0,6*	0,8	7	0,76	0,79	8	0,6	0,8	10	0,72	0,81
ZcwE04	5	0,59	0,54	3	0,44	0,36	2	0,29	0,49	5	0,55	0,52	2	0,29	0,49	5	0,48	0,54
ZcwG04	9	0,93	0,84	6	0,55	0,72	9	0,71	0,83	11	0,84	0,85	9	0,71	0,83	12	0,81	0,87
ZcwF07	7	0,86	0,84	7	0,78	0,82	5	0,43	0,62	9	0,84	0,85	5	0,43	0,62	9	0,73	0,83
ZcwE12	9	0,79	0,71	6	0,44	0,8	5	0,33*	0,64	10	0,71	0,75	5	0,33*	0,64	10	0,60*	0,81
Hg8.10	13	0,79	0,88	7	0,89	0,8	6	0,79	0,67	13	0,82	0,87	6	0,79	0,67	14	0,81	0,86
Pvce	7	0,27*	0,7	4	0,63	0,67	3	0*	0,6	7	0,35*	0,7	3	0*	0,6	7	0,25*	0,7
M11	7	0,79	0,78	5	0,71	0,73	7	0,79	0,8	7	0,77	0,77	7	0,79	0,8	9	0,78	0,81
Pv9	8	0,9	0,84	5	0,86	0,76	5	0,62	0,7	9	0,89	0,85	5	0,62	0,7	9	0,82	0,84
Hg6.3	5	0,41*	0,61	4	0,75	0,65	4	0,5	0,52	6	0,49	0,62	4	0,5	0,52	7	0,48*	0,72

Table 6: Pairwise F-statistics among sampling localities and between clusters. **P<0,05*

		STR		
		Peru	Chile	Atlantic
Chile	Fst	0.02		
	Rst	0.09*		
Brazil	Fst	0.11*	0.11*	
	Rst	0.17*	0.06	
Pacific	Fst			0.10*
	Rst			0.13*

CONCLUSÕES GERAIS

Os resultados do presente trabalho demonstram uma antiga e forte separação entre as populações dos oceanos Atlântico e Pacífico. Esta forte estruturação indica a influência contínua de eventos geológicos e/ou geoclimáticos na história evolutiva da espécie que possivelmente geraram o padrão de separação encontrado até hoje entre os oceanos. Tal padrão pode ser compartilhado por outras espécies de distribuição semelhante. Desta forma, trabalhos futuros de filogeografia de outras espécies que tenham distribuição ao longo da costa atlântica e pacífica, como *Arctocephalus australis*, ajudarão a definir melhor quais eventos geológicos e/ou ecológicos podem ter influenciado na história evolutiva e estruturação da espécie. As diferenças encontradas nos resultados dos diferentes marcadores indicam filopatria de fêmeas e fluxo gênico mediado por machos, um padrão já descrito para otarideos e característico de um comportamento reprodutivo poligínico.

Apesar de ter sido extremamente caçada nos últimos séculos, nossos resultados mostram que a caça não diminuiu a diversidade genética da espécie. Na verdade, o Leão-marinho-do-sul vem evoluindo com tamanho efetivo constante desde a era pleistocênica até os dias atuais. Existe ainda a possibilidade de eventos climáticos sucessivos, como o *El niño,* terem influenciado na dinâmica populacional da população do Pacífico, como vem sendo proposto por outros pesquisadores. No entanto, o grande tamanho populacional e a ampla distribuição fazem com que eventos estocásticos regionais tenham pouca influencia na espécie como um todo. Estas características possibilitam manejo controlado da espécie sem que haja riscos genéticos.

Certas questões interessantes como a quantificação do padrão de dispersão de machos ainda precisam ser respondidas. Particularidades nos padrões filogeográficos dentro

de cada oceano e localização mais exata da barreira entre os stocks são igualmente relevantes. Além disso, do ponto de vista taxonômico, nossos resultados demonstram a existência de duas unidades evolutivamente significativas (ESU) dentro da espécie, e talvez merecessem ser tratadas como subspécies. Para responder todas essas questões é necessário amostrar áreas no extremo sul do continente americano e talvez utilizar ainda outros marcadores nucleares, como íntrons e/ou regiões intergênicas e marcadores do cromossomo Y.

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